PCT10

RAW SEQUENCE LISTING
PATENT APPLICATION: US/10/009,500A

DATE: 12/11/2002
TIME: 09:28:55

Input Set : N:\Crf4\11182002\J009500.raw
Output Set: N:\CRF4\12112002\J009500A.raw

```
 1 <110> APPLICANT: KORDOWICZ, MARIA
 2        GUESSOW, DETLET
 3        HOFMANN, UWE
 4        PACUSZKA, TADEUSZ
 5        GARDAS, ANDRZEJ
 6 <120> TITLE OF INVENTION: HYALURONIDASE FROM THE HIRUDINARIA MANILLENSIS,
 7        ISOLATION, PURIFICATION AND RECOMBINANT METHOD OF
 8        PRODUCTION
 9 <130> FILE REFERENCE: MERCK 2332
10 <140> CURRENT APPLICATION NUMBER: US/10/009,500A
11 <141> CURRENT FILING DATE: 2002-04-08
12 <160> NUMBER OF SEQ ID NOS: 20
13 <170> SOFTWARE: PatentIn Ver. 2.1
15 <210> SEQ ID NO: 1
16 <211> LENGTH: 488
17 <212> TYPE: PRT
18 <213> ORGANISM: Hirudinaria manillensis
19 <400> SEQUENCE: 1
20        Lys Glu Ile Ala Val Thr Ile Asp Asp Lys Asn Val Ile Ala Ser Val
21         1               5                  10                  15
22        Ser Glu Ser Phe His Gly Val Ala Phe Asp Ala Ser Leu Phe Ser Pro
23                        20                  25                  30
24        Lys Gly Leu Trp Ser Phe Val Asp Ile Thr Ser Pro Lys Leu Phe Lys
25                    35                  40                  45
26        Leu Leu Glu Gly Leu Ser Pro Gly Tyr Phe Arg Val Gly Gly Thr Phe
27                50                  55                  60
28        Ala Asn Trp Leu Phe Phe Asp Leu Asp Glu Asn Asn Lys Trp Lys Asp
29         65                  70                  75                  80
30        Tyr Trp Ala Phe Lys Asp Lys Thr Pro Glu Thr Ala Thr Ile Thr Arg
31                        85                  90                  95
32        Arg Trp Leu Phe Arg Lys Gln Asn Asn Leu Lys Lys Glu Thr Glu Asp
33                        100                 105                 110
34        Asp Leu Val Lys Leu Thr Lys Gly Ser Lys Met Arg Leu Leu Phe Asp
35                    115                 120                 125
36        Leu Asn Ala Glu Val Arg Thr Gly Tyr Glu Ile Gly Lys Lys Met Thr
37                130                 135                 140
38        Ser Thr Trp Asp Ser Ser Glu Ala Glu Lys Leu Phe Lys Tyr Cys Val
39         145                 150                 155                 160
40        Ser Lys Gly Tyr Gly Asp Asn Ile Asp Trp Glu Leu Gly Asn Glu Pro
41                        165                 170                 175
42        Asp His Thr Ser Ala His Asn Leu Thr Glu Lys Gln Val Gly Glu Asp
43                        180                 185                 190
44        Phe Lys Ala Leu His Lys Val Leu Glu Lys Tyr Pro Thr Leu Asn Lys
```

Does Not Comply
Corrected Diskette Needed

file://C:\CRF4\Outhold\VsrJ009500A.htm

RAW SEQUENCE LISTING
PATENT APPLICATION: US/10/009,500A

DATE: 12/11/2002
TIME: 09:28:55

Input Set : **N:\Crf4\11182002\J009500.raw**
Output Set: **N:\CRF4\12112002\J009500A.raw**

```
45                 195                 200                 205
46     Gly Ser Leu Val Gly Pro Asp Val Gly Trp Met Gly Val Ser Tyr Val
47         210                 215                 220
48     Lys Gly Leu Ala Asp Gly Ala Gly Asp Leu Val Thr Ala Phe Thr Leu
49     225                 230                 235                 240
50     His Gln Tyr Tyr Phe Asp Gly Asn Thr Ser Asp Val Ser Thr Tyr Leu
51                 245                 250                 255
52     Asp Ala Thr Tyr Phe Lys Lys Leu Gln Gln Leu Phe Asp Lys Val Lys
53                 260                 265                 270
54     Asp Val Leu Lys Asn Ser Gln His Lys Asp Lys Pro Leu Trp Leu Gly
55                 275                 280                 285
56     Glu Thr Ser Ser Gly Tyr Asn Ser Gly Thr Lys Asp Val Ser Asp Arg
57         290                 295                 300
58     Tyr Val Ser Gly Phe Leu Thr Leu Asp Lys Leu Gly Leu Ser Ala Ala
59     305                 310                 315                 320
60     Asn Asn Val Lys Val Val Ile Arg Gln Thr Ile Tyr Asn Gly Tyr Tyr
61                 325                 330                 335
62     Gly Leu Leu Asp Lys Asn Thr Leu Glu Pro Asn Pro Asp Tyr Trp Leu
63                 340                 345                 350
64     Met His Val His Asn Ser Leu Val Gly Asn Thr Val Phe Lys Val Asp
65         355                 360                 365
66     Val Ser Asp Pro Thr Asn Lys Ala Arg Val Tyr Ala Gln Cys Thr Lys
67         370                 375                 380
68     Thr Asn Ser Lys His Thr Gln Ser Arg Tyr Tyr Lys Gly Ser Leu Thr
69     385                 390                 395                 400
70     Ile Phe Ala Leu Asn Val Gly Asp Glu Asp Val Thr Leu Lys Ile Asp
71                 405                 410                 415
72     Gln Tyr Gly Gly Lys Lys Ile Tyr Ser Tyr Ile Leu Thr Pro Glu Gly
73                 420                 425                 430
74     Gly Gln Leu Thr Ser Gln Lys Val Leu Leu Asn Gly Lys Glu Leu Lys
75                 435                 440                 445
76     Leu Val Ser Asp Gln Leu Pro Glu Leu Asn Ala Asn Glu Ser Lys Thr
77         450                 455                 460
78     Ser Phe Thr Leu Ser Pro Lys Thr Phe Gly Phe Phe Val Val Ser Asp
79     465                 470                 475                 480
80     Ala Asn Val Glu Ala Cys Lys Lys
81                 485
83  <210> SEQ ID NO: 2
84  <211> LENGTH: 1464
85  <212> TYPE: DNA
86  <213> ORGANISM: Hirudinaria manillensis
87  <220> FEATURE:
88  <221> NAME/KEY: CDS
89  <222> LOCATION: (1)..(1464)
90  <400> SEQUENCE: 2
91     aaa gag att gcc gtg aca att gac gat aag aat gtg att gca tct gcc    48
92     Lys Glu Ile Ala Val Thr Ile Asp Asp Lys Asn Val Ile Ala Ser Ala
93      1               5                  10                  15
94     agt ggg tct ttc ctt gga gtt gcc ttt gat gcg tct cta ttt tcg ccc    96
```

RAW SEQUENCE LISTING   DATE: 12/11/2002
PATENT APPLICATION: US/10/009,500A   TIME: 09:28:55

Input Set : **N:\Crf4\11182002\J009500.raw**
Output Set: **N:\CRF4\12112002\J009500A.raw**

```
95     Ser Gly Ser Phe Leu Gly Val Ala Phe Asp Ala Ser Leu Phe Ser Pro
96                   20                  25                  30
97     aag ggt ctt tgg agc ttt gtt gat att acc tct cca aaa ttg ttc aaa   144
98     Lys Gly Leu Trp Ser Phe Val Asp Ile Thr Ser Pro Lys Leu Phe Lys
99                   35                  40                  45
100    ttg ctg gaa gga ctt tct cct gga tac ttc agg gtt ggc gga acg ttt   192
101    Leu Leu Glu Gly Leu Ser Pro Gly Tyr Phe Arg Val Gly Gly Thr Phe
102               50                  55                  60
103    gcc aat tgg ctg ttt ttt gac ttg gac gaa aat aat aag tgg aag gat   240
104    Ala Asn Trp Leu Phe Phe Asp Leu Asp Glu Asn Asn Lys Trp Lys Asp
105            65                  70                  75                  80
106    tat tgg gct ttt aaa gac aaa acc ccc gaa act gcg aca ata aca agg   288
107    Tyr Trp Ala Phe Lys Asp Lys Thr Pro Glu Thr Ala Thr Ile Thr Arg
108                   85                  90                  95
109    aga tgg ctg ttc aga aaa caa aat aat ctg aaa aag gag act ttt gac   336
110    Arg Trp Leu Phe Arg Lys Gln Asn Asn Leu Lys Lys Glu Thr Phe Asp
111                   100                 105                 110
112    aat tta gtg aaa cta aca aag gga agc aag atg aga ttg tta ttc gat   384
113    Asn Leu Val Lys Leu Thr Lys Gly Ser Lys Met Arg Leu Leu Phe Asp
114                   115                 120                 125
115    ttg aat gcc gaa gtg agc act ggt tat gaa att gga aag aag atg aca   432
116    Leu Asn Ala Glu Val Arg Thr Gly Tyr Glu Ile Gly Lys Lys Met Thr
117                   130                 135                 140
118    tcc act tgg gat tca tcg gag gct gaa aag tta ttt aaa tat tgt gtg   480
119    Ser Thr Trp Asp Ser Ser Glu Ala Glu Lys Leu Phe Lys Tyr Cys Val
120    145                 150                 155                 160
121    tca aaa ggt tac gga gac aat atc gat tgg gaa ctt gga aat gaa ccg   528
122    Ser Lys Gly Tyr Gly Asp Asn Ile Asp Trp Glu Leu Gly Asn Glu Pro
123                   165                 170                 175
124    gac cac acc tca gct cac aat tta act gaa aag cag gtt gga gaa gat   576
125    Asp His Thr Ser Ala His Asn Leu Thr Glu Lys Gln Val Gly Glu Asp
126                   180                 185                 190
127    ttt aaa gca ctg cat aaa gtt cta gag aaa tat cca act ctt aac aag   624
128    Phe Lys Ala Leu His Lys Val Leu Glu Lys Tyr Pro Thr Leu Asn Lys
129                   195                 200                 205
130    gga tcg ctc gtt ggt cca gat gta ggg tgg atg ggc gtc agt wac gtc   672
131    Gly Ser Leu Val Gly Pro Asp Val Gly Trp Met Gly Val Ser Tyr Val
132               210                 215                 220
133    aag gga ttg gca gac gag gcr ggt gac cat gta ack gct ttt aca ctc   720
134    Lys Gly Leu Ala Asp Glu Ala Gly Asp His Val Thr Ala Phe Thr Leu
135    225                 230                 235                 240
136    cac caa tat tat ttc gat gga aac acy tct gat gta tca ata tat ctt   768
137    His Gln Tyr Tyr Phe Asp Gly Asn Thr Ser Asp Val Ser Ile Tyr Leu
138               245                 250                 255
139    gat gcc aca tac ttt aag aag ctg caa caa cta ttt gat aaa gtg aaa   816
140    Asp Ala Thr Tyr Phe Lys Lys Leu Gln Gln Leu Phe Asp Lys Val Lys
141               260                 265                 270
142    gat gtt ttg aaa gat tct cca cat aaa gac gaa cca tta tgg ctt gga   864
143    Asp Val Leu Lys Asp Ser Pro His Lys Asp Glu Pro Leu Trp Leu Gly
```

RAW SEQUENCE LISTING

DATE: 12/11/2002

PATENT APPLICATION: US/10/009,500A

TIME: 09:28:55

Input Set : N:\Crf4\11182002\J009500.raw
Output Set: N:\CRF4\12112002\J009500A.raw

```
144          275              280              285
145     gaa aca agt tct gga tac aac agc ggc aca gaa gat gta tcc gat cga   912
146     Glu Thr Ser Ser Gly Tyr Asn Ser Gly Thr Glu Asp Val Ser Asp Arg
147          290              295              300
148     tat gtt tca gga ttt cta aca tta gac aag ttg ggt ctc agt gca gcc   960
149     Tyr Val Ser Gly Phe Leu Thr Leu Asp Lys Leu Gly Leu Ser Ala Ala
150     305              310              315              320
151     aac aat gta aag gtt gtt ata aga cag aca ata tac aat gga tat tat   1008
152     Asn Asn Val Lys Val Val Ile Arg Gln Thr Ile Tyr Asn Gly Tyr Tyr
153                   325              330              335
154     ggt ctc ctt gac aaa aac act tta gag ccg aat ccg gat tac tgg tta   1056
155     Gly Leu Leu Asp Lys Asn Thr Leu Glu Pro Asn Pro Asp Tyr Trp Leu
156               340              345              350
157     atg cat gtt cat aat tct ttg gtc gga aat aca gtt ttt aaa gtt gac   1104
158     Met His Val His Asn Ser Leu Val Gly Asn Thr Val Phe Lys Val Asp
159               355              360              365
160     gtt agt gat cca act aat aaa gca aga gtt tac gcg caa tgt acc aaa   1152
161     Val Ser Asp Pro Thr Asn Lys Ala Arg Val Tyr Ala Gln Cys Thr Lys
162          370              375              380
163     aca aat agc aaa cat act caa agc aga tat tac aag ggc tct ttg aca   1200
164     Thr Asn Ser Lys His Thr Gln Ser Arg Tyr Tyr Lys Gly Ser Leu Thr
165     385              390              395              400
166     atc ttt gca ctt aat gtt gga gat gga gat gta acg tta aag atc ggt   1248
167     Ile Phe Ala Leu Asn Val Gly Asp Gly Asp Val Thr Leu Lys Ile Gly
168                   405              410              415
169     caa tac agc ggt aaa aaa att tat tca tac att ctg aca cct gaa gga   1296
170     Gln Tyr Ser Gly Lys Lys Ile Tyr Ser Tyr Ile Leu Thr Pro Glu Gly
171               420              425              430
172     gga caa ctt aca tca cag aaa gtt ctc ttg aat gga aag gaa ttg aac   1344
173     Gly Gln Leu Thr Ser Gln Lys Val Leu Leu Asn Gly Lys Glu Leu Asn
174               435              440              445
175     tta gtg tct gat cag tta cca gaa cta aat gca gat gaa tcc aaa aca   1392
176     Leu Val Ser Asp Gln Leu Pro Glu Leu Asn Ala Asp Glu Ser Lys Thr
177          450              455              460
178     tct ttc acc tta tcc cca aag aca ttt ggt ttt ttt gtt gtt tcc gat   1440
179     Ser Phe Thr Leu Ser Pro Lys Thr Phe Gly Phe Phe Val Val Ser Asp
180     465              470              475              480
181     gct aat gtt gaa gca tgy aar aar                                   1464
182     Ala Asn Val Glu Ala Cys Lys Lys
183                   485
```

185 <210> SEQ ID NO: 3
186 <211> LENGTH: 488
187 <212> TYPE: PRT
188 <213> ORGANISM: Hirudinaria manillensis
189 <220> FEATURE:
190 <221> NAME/KEY: variation
191 <222> LOCATION: (223)
192 <223> OTHER INFORMATION: (May be Asn)
193 <400> SEQUENCE: 3

(handwritten annotations:)
Please Correct any
Similar Error
in Subsequent Sequences

(See p-5)
"Tyr" is at location 223. Tyr can
only represent itself nothing
else. Xaa and Explains in
use L2207 - L2237 section.

RAW SEQUENCE LISTING
PATENT APPLICATION: US/10/009,500A

DATE: 12/11/2002
TIME: 09:28:55

Input Set : N:\Crf4\11182002\J009500.raw
Output Set: N:\CRF4\12112002\J009500A.raw

194 | Lys | Glu | Ile | Ala | Val | Thr | Ile | Asp | Asp | Lys | Asn | Val | Ile | Ala | Ser | Ala
195 | 1 | | | | 5 | | | | | 10 | | | | | 15 |
196 | Ser | Gly | Ser | Phe | Leu | Gly | Val | Ala | Phe | Asp | Ala | Ser | Leu | Phe | Ser | Pro
197 | | | 20 | | | | | 25 | | | | | 30 | | |
198 | Lys | Gly | Leu | Trp | Ser | Phe | Val | Asp | Ile | Thr | Ser | Pro | Lys | Leu | Phe | Lys
199 | | | 35 | | | | | 40 | | | | | 45 | | |
200 | Leu | Leu | Glu | Gly | Leu | Ser | Pro | Gly | Tyr | Phe | Arg | Val | Gly | Gly | Thr | Phe
201 | | 50 | | | | | 55 | | | | | 60 | | | |
202 | Ala | Asn | Trp | Leu | Phe | Phe | Asp | Leu | Asp | Glu | Asn | Asn | Lys | Trp | Lys | Asp
203 | 65 | | | | | 70 | | | | | 75 | | | | | 80
204 | Tyr | Trp | Ala | Phe | Lys | Asp | Lys | Thr | Pro | Glu | Thr | Ala | Thr | Ile | Thr | Arg
205 | | | | | 85 | | | | | 90 | | | | | 95 |
206 | Arg | Trp | Leu | Phe | Arg | Lys | Gln | Asn | Asn | Leu | Lys | Lys | Glu | Thr | Phe | Asp
207 | | | | 100 | | | | | 105 | | | | | 110 | |
208 | Asn | Leu | Val | Lys | Leu | Thr | Lys | Gly | Ser | Lys | Met | Arg | Leu | Leu | Phe | Asp
209 | | | 115 | | | | | 120 | | | | | 125 | | |
210 | Leu | Asn | Ala | Glu | Val | Arg | Thr | Gly | Tyr | Glu | Ile | Gly | Lys | Lys | Met | Thr
211 | | 130 | | | | | 135 | | | | | 140 | | | |
212 | Ser | Thr | Trp | Asp | Ser | Ser | Glu | Ala | Glu | Lys | Leu | Phe | Lys | Tyr | Cys | Val
213 | 145 | | | | | 150 | | | | | 155 | | | | | 160
214 | Ser | Lys | Gly | Tyr | Gly | Asp | Asn | Ile | Asp | Trp | Glu | Leu | Gly | Asn | Glu | Pro
215 | | | | | 165 | | | | | 170 | | | | | 175 |
216 | Asp | His | Thr | Ser | Ala | His | Asn | Leu | Thr | Glu | Lys | Gln | Val | Gly | Glu | Asp
217 | | | | 180 | | | | | 185 | | | | | 190 | |
218 | Phe | Lys | Ala | Leu | His | Lys | Val | Leu | Glu | Lys | Tyr | Pro | Thr | Leu | Asn | Lys
219 | | | 195 | | | | | 200 | | | | | 205 | | |
220 | Gly | Ser | Leu | Val | Gly | Pro | Asp | Val | Gly | Trp | Met | Gly | Val | Ser | (Tyr) | Val
221 | | | 210 | | | | | 215 | | | | | 220 | | |
222 | Lys | Gly | Leu | Ala | Asp | Glu | Ala | Gly | Asp | His | Val | Thr | Ala | Phe | Thr | Leu
223 | 225 | | | | | 230 | | | | | 235 | | | | | 240
224 | His | Gln | Tyr | Tyr | Phe | Asp | Gly | Asn | Thr | Ser | Asp | Val | Ser | Ile | Tyr | Leu
225 | | | | | 245 | | | | | 250 | | | | | 255 |
226 | Asp | Ala | Thr | Tyr | Phe | Lys | Lys | Leu | Gln | Gln | Leu | Phe | Asp | Lys | Val | Lys
227 | | | | 260 | | | | | 265 | | | | | 270 | |
228 | Asp | Val | Leu | Lys | Asp | Ser | Pro | His | Lys | Asp | Glu | Pro | Leu | Trp | Leu | Gly
229 | | | 275 | | | | | 280 | | | | | 285 | | |
230 | Glu | Thr | Ser | Ser | Gly | Tyr | Asn | Ser | Gly | Thr | Glu | Asp | Val | Ser | Asp | Arg
231 | | | 290 | | | | | 295 | | | | | 300 | | |
232 | Tyr | Val | Ser | Gly | Phe | Leu | Thr | Leu | Asp | Lys | Leu | Gly | Leu | Ser | Ala | Ala
233 | 305 | | | | | 310 | | | | | 315 | | | | | 320
234 | Asn | Asn | Val | Lys | Val | Val | Ile | Arg | Gln | Thr | Ile | Tyr | Asn | Gly | Tyr | Tyr
235 | | | | | 325 | | | | | 330 | | | | | 335 |
236 | Gly | Leu | Leu | Asp | Lys | Asn | Thr | Leu | Glu | Pro | Asn | Pro | Asp | Tyr | Trp | Leu
237 | | | | 340 | | | | | 345 | | | | | 350 | |
238 | Met | His | Val | His | Asn | Ser | Leu | Val | Gly | Asn | Thr | Val | Phe | Lys | Val | Asp
239 | | | 355 | | | | | 360 | | | | | 365 | | |
240 | Val | Ser | Asp | Pro | Thr | Asn | Lys | Ala | Arg | Val | Tyr | Ala | Gln | Cys | Thr | Lys
241 | | | 370 | | | | | 375 | | | | | 380 | | |
242 | Thr | Asn | Ser | Lys | His | Thr | Gln | Ser | Arg | Tyr | Tyr | Lys | Gly | Ser | Leu | Thr

VERIFICATION SUMMARY
PATENT APPLICATION: US/10/009,500A

DATE: 12/11/2002
TIME: 09:28:56

Input Set : N:\Crf4\11182002\J009500.raw
Output Set: N:\CRF4\12112002\J009500A.raw